

Ovarian Hyperemia Pregnancy Test

PHILIP M. SAVAGE, JR., M.D., and GLORIA VALERIO, M.T., San Bernardino

THE FIRST ACCURATE DIAGNOSTIC test for pregnancy was outlined in 1928 by Aschheim and Zondek¹ after they demonstrated the presence of a gonad-stimulating substance in the blood and urine of pregnant women. The Aschheim-Zondek test is based on the fact that this gonad-stimulating substance (which we now know is chorionic gonadotropin) when injected subcutaneously into immature female mice, produces hemorrhagic follicles in the ovaries.

Since the original meritorious work of Aschheim and Zondek, the hormonal test for the diagnosis of pregnancy has become one of the most talked about subjects in medical journals. Most of the earlier reports were corroborations of the value of the test. However, since the Aschheim-Zondek technique requires the use of five mice and takes 96 hours, many investigators began to modify the original test to make it more adaptable to routine clinical use.

Most workers in the field have elected to use immature rats instead of mice. The first successful pregnancy test in which rats were used and results were known in 24 hours was reported by Kelso⁴ in 1940. In 1941 Frank and coworkers³ shortened the procedure to from 8 to 24 hours. The following year Salmon and his coworkers⁶ reported a six-hour ovarian hyperemia pregnancy test, and in 1943 Kupperman and his associates⁵ suggested that the end-point of the test be shortened to two hours.

On the other hand, investigations by Bunde² and by Zondek and Sulman⁷ in 1947 demonstrated that shortening the time of the test to less than 24 hours renders it less reliable.

The ovarian hyperemia pregnancy test has been used at the laboratory of the San Bernardino Medical Group since August 1957.

The method employed uses two immature female rats, Wistar strain, age 22 to 30 days, optimum weight 40 to 60 grams. Each rat receives a subcutaneous injection of 1 cc. of serum from the woman being tested. A second injection of 1 cc. of serum is administered after a time lapse of four hours.

Autopsy is performed the following day (approximately 18 hours after the first injection). The animals are killed by chloroform asphyxiation, and the abdomen is opened to expose the ovaries, which are examined immediately. If the ovaries are grossly

• In 310 cases in which a pregnancy test was carried out by injecting serum from the woman into immature female rats and observing the ovaries at autopsy 18 hours later, there were no false positive reactions. Results in three instances were false negative and in two of the three a positive reaction was obtained upon repeat of the test two weeks later.

The advantages of using serum for the test are believed to outweigh the convenience of obtaining urine specimens.

enlarged and hyperemic, with small hemorrhages visible on the surface, the result is positive for pregnancy; if the ovaries are small, pale and cream-colored (occasionally faintly pink) the result is negative.

If a physician requests a report sooner than 18 hours, autopsy is done at six to eight hours after the first injection. In this case, two additional rats are injected, making a total of four rats used for the test. If the hyperemic end-point is observed at the earlier autopsy, the test is reported as positive. A negative result at six to eight hours is not considered conclusive, and the final result of the test is not reported until the autopsy is done on the alternate pair of rats the following day. It has seemed to us, from both the clinical and psychological aspects, that the greatest possible accuracy is more important than speed. For this reason, we prefer the 18-hour test.

RESULTS

A statistical evaluation has been made from data on the first 315 ovarian hyperemia pregnancy tests carried out at the San Bernardino Medical Group.

The clinical course of the patient was followed closely in 310 of these cases; in five cases the clinical outcome is not known.

Of the 310 specimens in cases in which the clinical data are whole, 170 were obtained from women in whom pregnancy was subsequently verified clinically and 140 were from women who were not pregnant.

There was no instance of a false positive reaction. In three instances the reaction was not positive although the woman was pregnant. Two weeks after the first test a second one was done in two cases and the results then were positive. In the remaining case retesting was not carried out.

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DISCUSSION

Although the majority of laboratories performing this test use urine as the material to be injected (voiding being easier than the venipuncture necessary for obtaining serum) we prefer to use serum in our laboratory. Unextracted urine is frequently toxic, as it may be when the patient is bleeding, has cystitis or has been taking various drugs. Also, if the patient has polyuria, even a first morning urine specimen may be very dilute, which would necessitate concentrating the specimen.

Since the definite end-point of the test consists of hyperemia of the ovaries, it is imperative that the ovaries be examined immediately after death. In performing the autopsy, it is necessary to avoid penetrating vital organs or injuring vessels in the abdominal wall, since blood in the abdominal cavity makes it difficult to detect the ovarian hyperemia.

Anyone planning to use this test should become familiar with the normal gross appearance of the ovaries of immature rats in order that accurate readings be made. It is advisable to start with a series of serum specimens from women known to be pregnant and to contrast the ovarian reactions induced by these specimens with the condition of the ovaries

of rats injected with serum from women known not to be pregnant. Also, until one becomes adept in reading the tests, a control animal, preferably a litter mate, should be killed simultaneously for comparison with the injected rats.

San Bernardino Medical Group, 1700 Waterman Avenue, San Bernardino (Savage, Jr.).

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